Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supporting Information

Unfolding Transition State of Ubiquitin with Charged Residues has Higher Energy than that with Hydrophobic Residues

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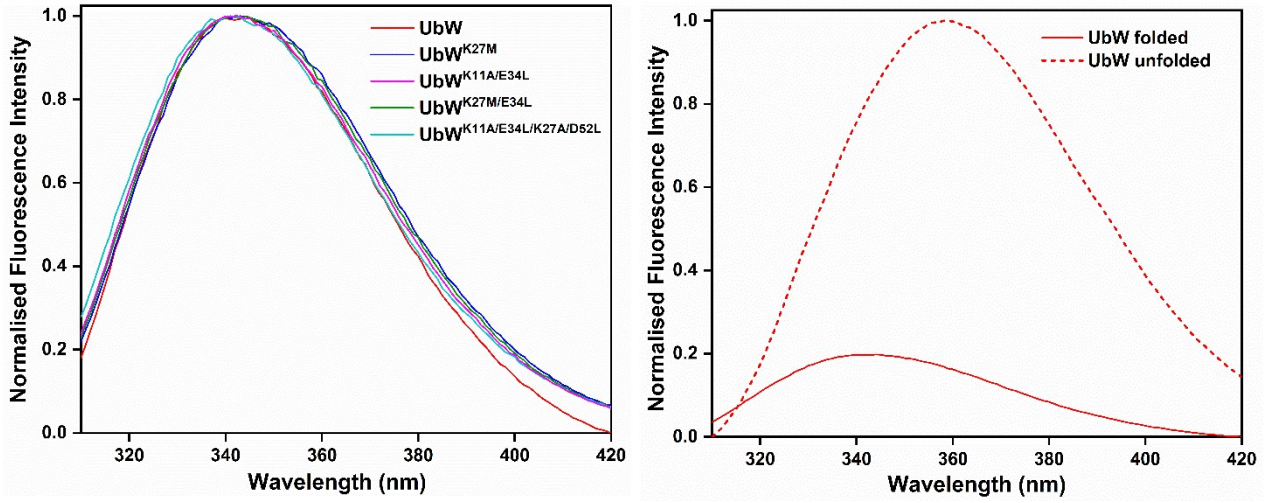

Fig. S1. Steady state fluorescence spectra of the ubiquitin variants. The tryptophan emission spectra of all the mutants are similar. The emission maxima for all the mutants are at ~340 nm suggesting similar tryptophan environment. The unfolded state spectra along with the folded state spectra for UbW are shown for comparison.

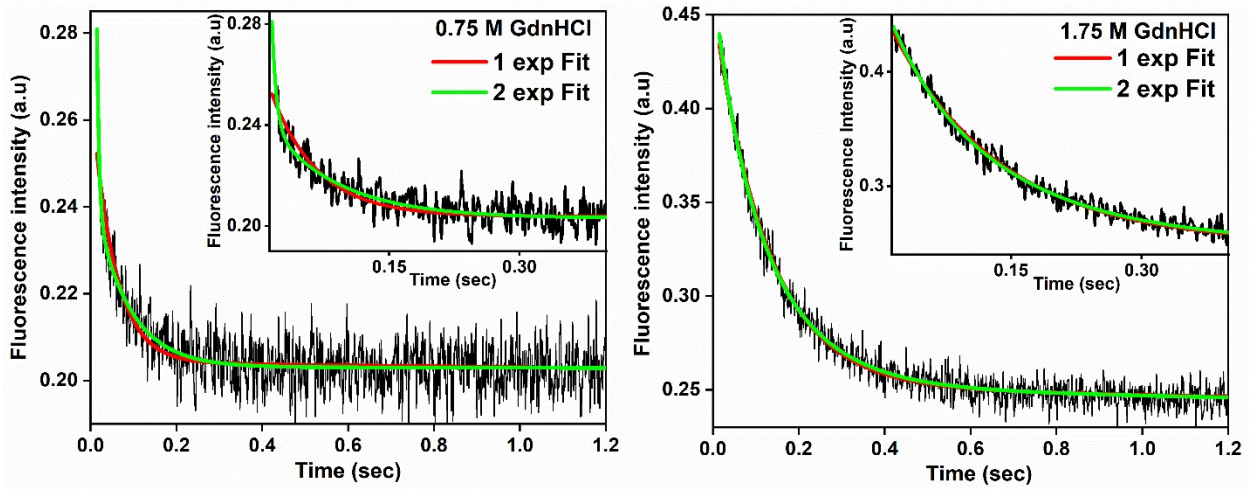

Fig. S2. Representative folding traces of UbW at 0.75 M GdnHCl and 1.75 M GdnHCl. The folding trace requires bi exponential fitting at 0.75 M GdnHCl. Single exponential fit does not capture the faster phase properly. At 1.75 M GdnHCl, the single and bi-exponential fits are almost identical and the major amplitude rate constant of the bi-exponential fit is similar to the single exponential rate constant.

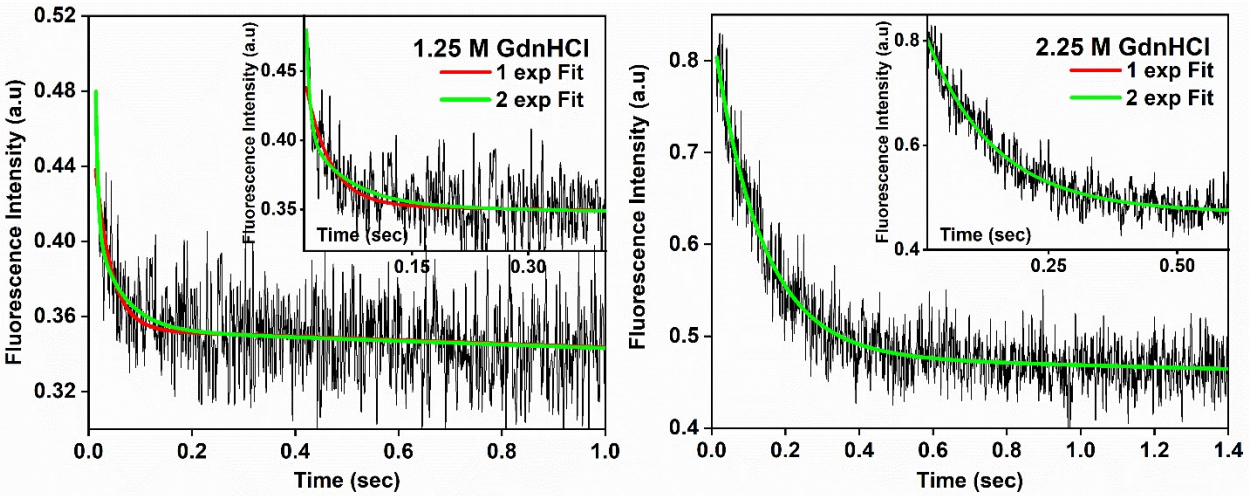

Fig. S3. Representative folding traces of UbW^{K27M} at 1.25 M GdnHCl and 2.25 M GdnHCl. The folding traces requires bi-exponential fitting for lower denaturant concentrations (shown for 1.25 M GdnHCl). The single and bi-exponential fits are almost identical at even higher concentrations of GdnHCl (shown for 2.25 M GdnHCl). The major amplitude rate constant obtained from the bi-exponential fits are similar to the single exponential ones.

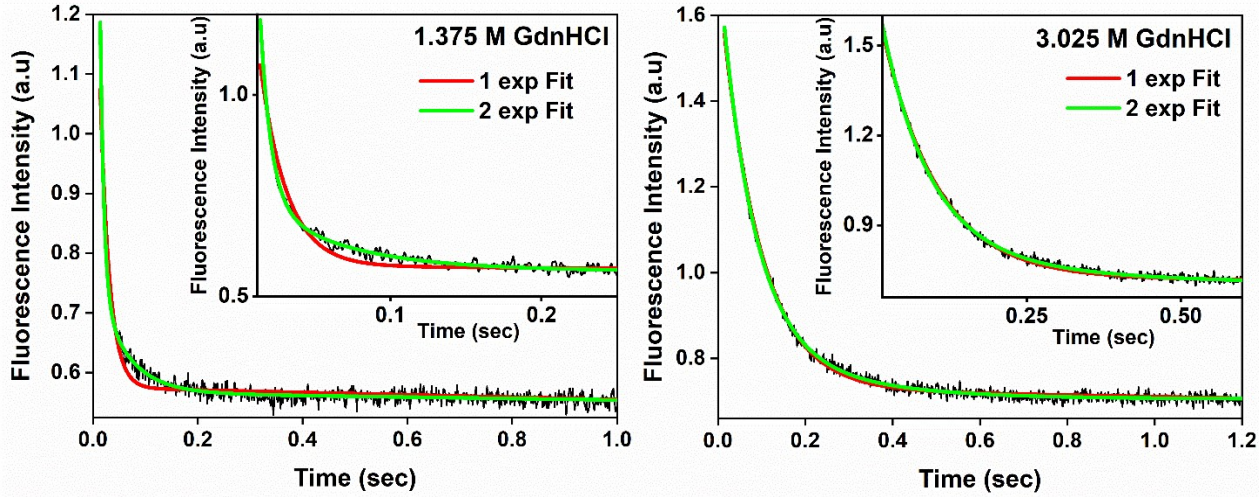

Fig. S4. Representative folding traces of $UbW^{K11A/E34L}$ at 1.375 M GdnHCl and 3.025 M GdnHCl. Folding traces required bi-exponential fits at lower denaturant concentrations (shown for 1.375 M GdnHCl) and the single and bi-exponential fits almost became identical at higher GdnHCl concentrations (Shown for 3.025 M GdnHCl).

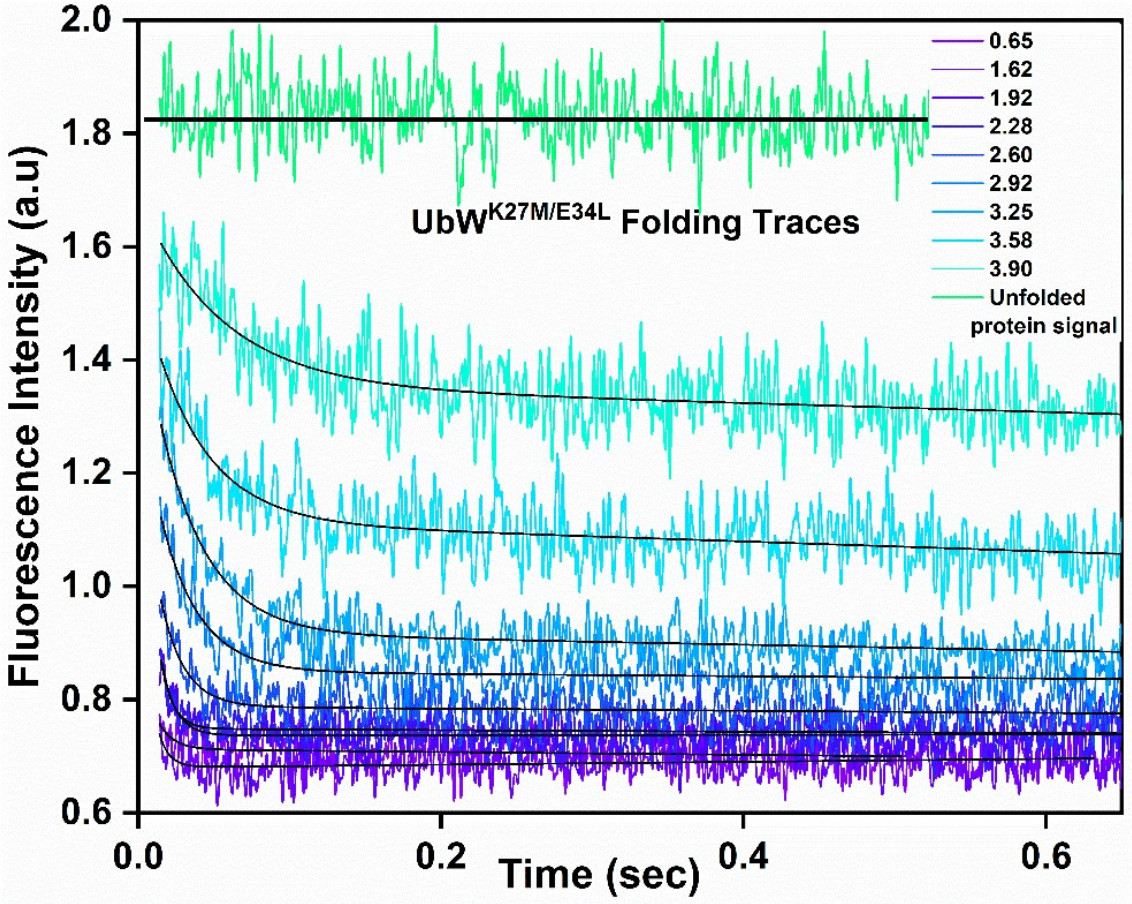

Fig. S5. Representative folding kinetic traces of $UbW^{K27M/E34L}$. The folding process was very fast for this protein as a result of which the faster phase could not be captured and the slower phase becomes the only observable phase. Very little signal change could be captured. The traces fit well to single exponential equations giving the slower rate constant. The GdnHCl concentrations are mentioned with their colour codes.

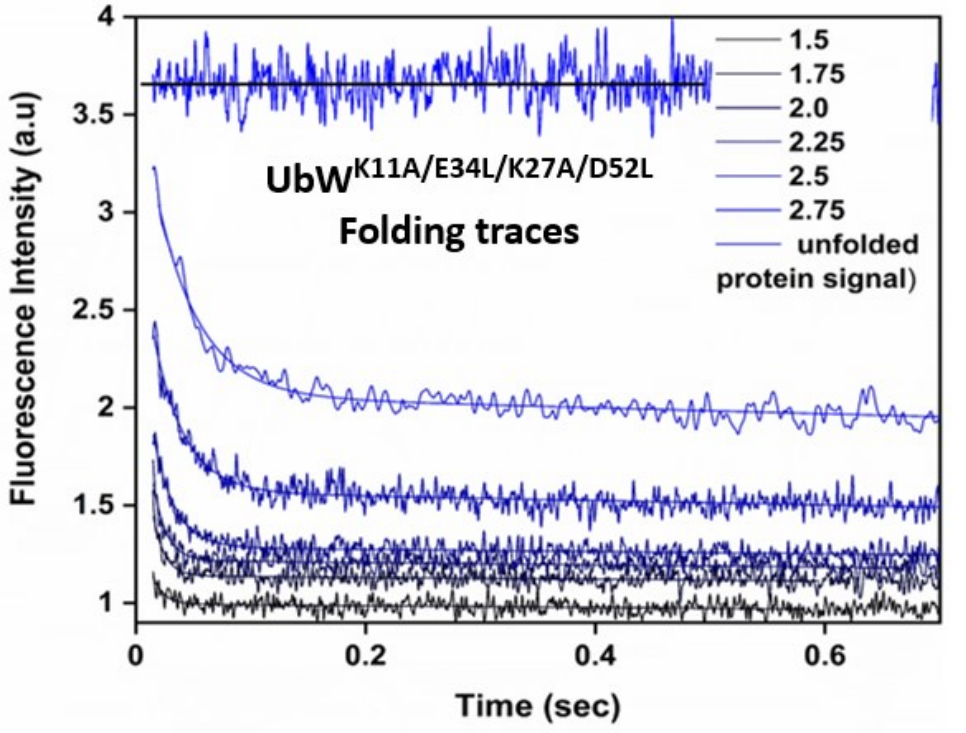

Fig. S6. Representative folding kinetic traces of $UbW^{K11A/E34L/K27A/D52L}$. The folding process was very fast for this protein as a result of which the faster phase could not be captured and the slower phase becomes the only observable phase. Very little signal change could be captured. The traces fit well to single exponential equations giving the slower rate constant. The GdnHCl concentrations are mentioned with their colour codes.

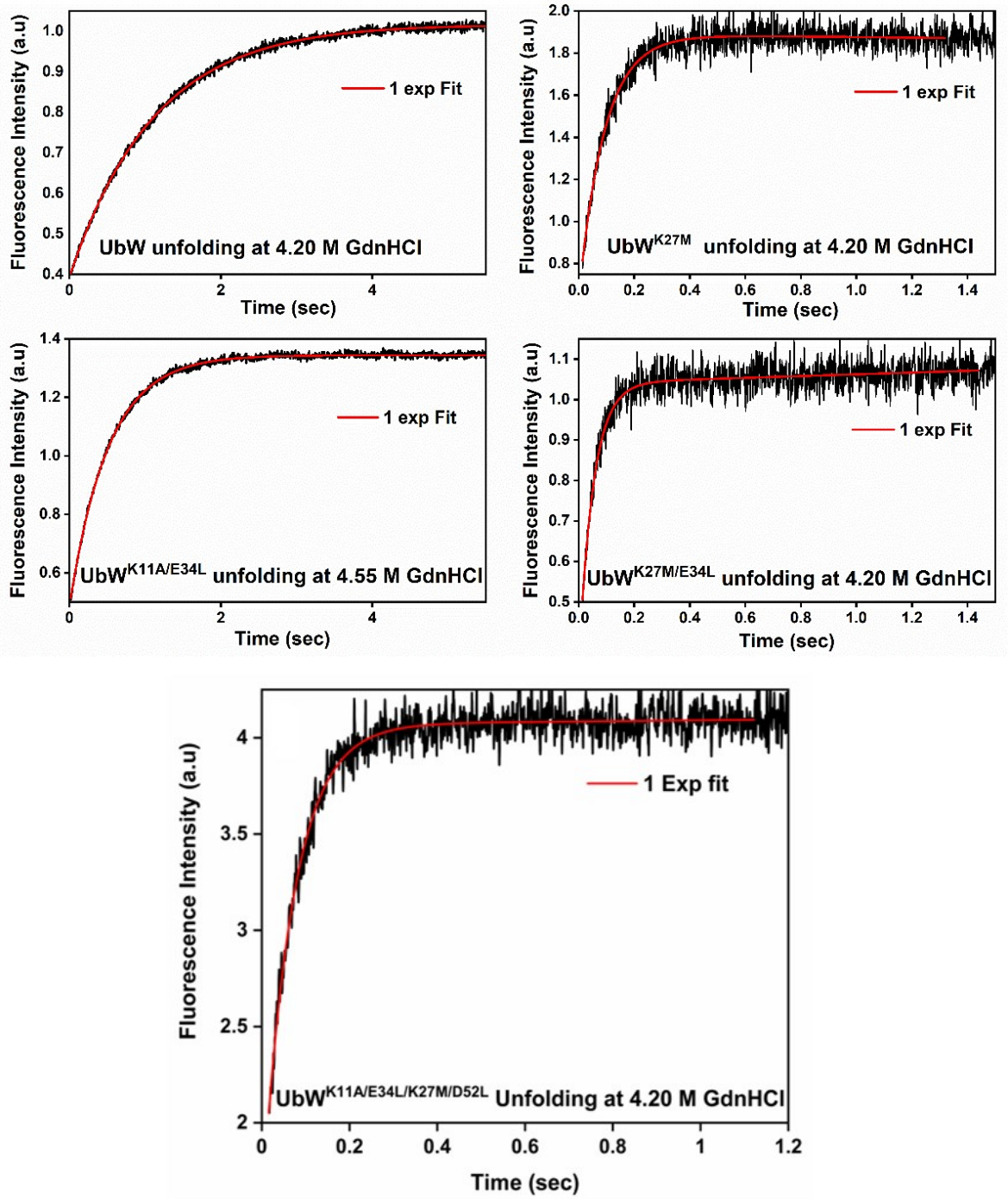

Fig. S7. Representative unfolding traces of the proteins. The unfolding traces could be very well fitted to single exponential equations. The unfolding process of UbW and UbW^{K11AE34L} is almost similar in terms of rate, and is much slower than the faster unfolding kinetics of UbW^{K27M} and UbW^{K27ME34L} and UbW$^{K11A/E34L/K27M/D52L}$.

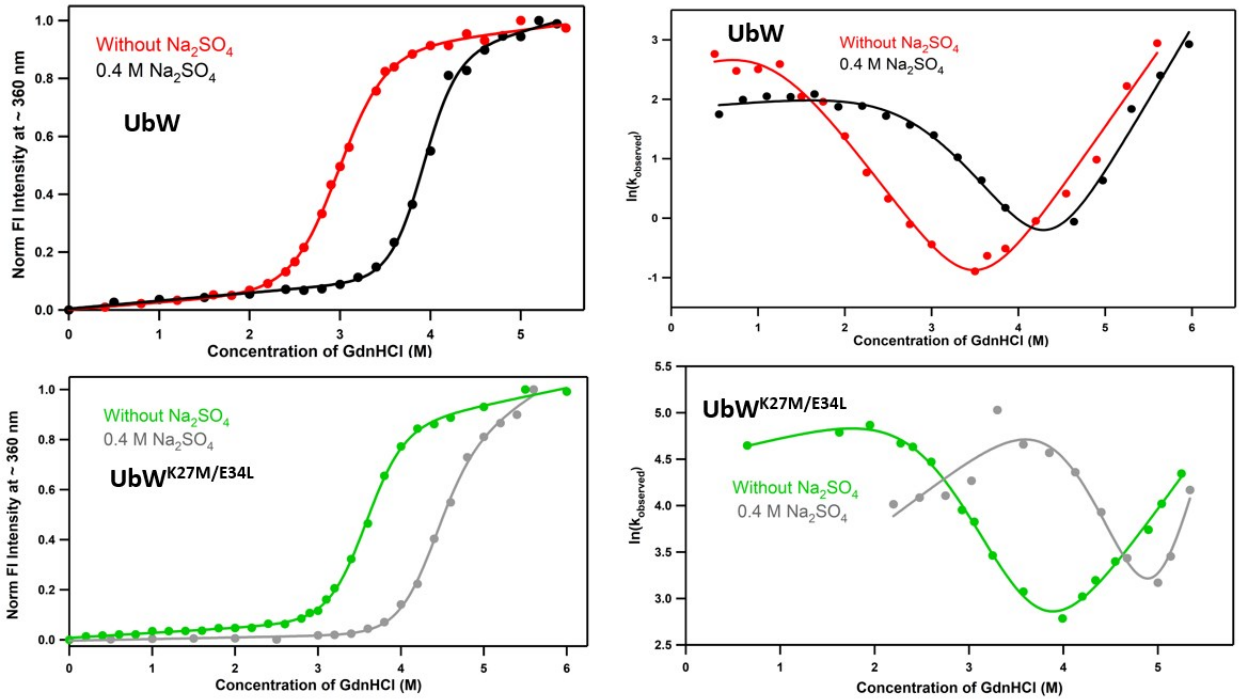

Fig. S8. Experiments with Na_2SO_4. The extent of increment in stability and the extent by which the chevron gets changed are similar for both UbW and $UbW^{K27M/E34L}$ in presence of Na_2SO_4